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Efficacy of chlorfenapyr against adult *Tribolium castaneum* exposed on concrete: effects of exposure interval, concentration and the presence of a food source after exposure

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Abstract Chlorfenapyr, an insecticidal pyrrole, was applied to concrete arenas at concentrations of 1.1, 0.825, 0.55, and 0.275 g of active ingredient [AI]/m^2. Adult *Tribolium castaneum* (Herbst), the red flour beetle, were exposed for 2, 4, or 8 h at each concentration, then removed and held either with or without food (wheat flour) for 7 days. Survival was assessed when the beetles were removed from the exposure arenas and daily during the post-exposure period. In the presence of food, survival was high regardless of concentration and the day on which post-treatment survival was assessed, but survival did decrease as the exposure period increased from 4 to 8 h. When the beetles were not given food after exposure, survival at each concentration and exposure period declined during the 1-week post-exposure assessments. This pattern of decrease could be described by linear and non-linear equations. Results show the presence of food material greatly compromised effectiveness of the insecticide, and emphasize the importance of cleaning and sanitation in conjunction with insecticide treatments.

Key words chlorfenapyr, efficacy, sanitation, *Tribolium castaneum*

Introduction

Chlorfenapyr is an insecticidal pyrrolle that inhibits adenosite triphosphate (ATP) production in the cellular structure of insects (McLeod *et al.*, 2002). It was first registered in the USA for control of termites, cockroaches and nuisance ants under the trade name Phantom®. The label was recently expanded to include food and feed mills, food handling areas, restaurants, and other areas where food is handled and stored, and stored-product insects have been added to the label for the USA. A previous test indicated effectiveness against both *Tribolium castaneum* Herbst, the red flour beetle, and *Tribolium confusum* (Jacquelin DuVal), the confused flour beetle, with *T. castaneum* being the more tolerant of the two species (Arthur, 2008).

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Tribolium castaneum is a world-wide pest commonly found in raw stored grains, milling facilities, and food warehouses (Rees, 2004). In many indoor facilities, the presence of food material through spillage and the manufacturing and milling processes offer harborage sites where insects can escape exposure to insecticides (Campbell & Arthur, 2007). Previous tests have also indicated that in the presence of a whole-wheat flour food source, survival of *T. castaneum* exposed to residual and contact insecticides often increases in comparison to survival of beetles exposed to the insecticides and not provided with food (Arthur, 1998b, 2000a, 2000b).

Residual insecticides can be applied to floors and walls inside mills and food storage sites as part of the insect pest management program. Insects come into contact with the insecticidal residues through movement on and absorption from the treated surface. Survival of *T. castaneum* (and most other stored-product insects) on a treated surface usually decreases in direct proportion to the actual time of exposure (Arthur, 1999), and consequently this increase in exposure period is comparable to an increase in the actual concentration of active ingredient of an insecticide. The objectives of this test were to determine: (i) if the presence of food would affect survival of *T. castaneum* after exposure on concrete treated with chlorfenapyr; and (ii) the effects of varying concentrations and exposure periods on beetle survival with and without food.

Materials and methods

Individual concrete exposure arenas were created as described by Arthur (2008) using the bottom portion of a standard plastic 100-mm Petri dish (62 cm^2 is the measured area of the bottom of the dish). A concrete patching material (Rockkite®) purchased from a local hardware store was mixed with water to create a slurry, and ≈ 10 mL of this slurry was poured into the bottom portion of the Petri dish. Insecticide spray solutions were formulated using chlorfenapyr (Phantom® Emulsifiable Concentrate [EC], 21.45% active ingredient [AI], 240 mg [AI]/mL). The label specifies 88 mL of product into 3 783 mL water to make a 0.5% diluted concentration. The maximum volume of spray selected for the individual arenas was based on the label rate of 35.5 mL per 0.182 m^2 of the 0.5% concentration. This is equivalent to 1.2 mL per the 62 cm^2 area of the individual treatment arenas, or 1.1 g [AI]/m^2. Four separate insecticide solutions were created for the four replicates at this maximum rate. An additional series of four solutions were created for each of four separate replicates treated at 0.9, 0.6, and 0.3 mL per the 62 cm^2 area of the individual treatment arenas, which gave measured concentrations of 0.825, 0.55, and 0.275 g [AI]/m^2, respectively.

The other factors evaluated with concentration were exposure periods of 2, 4, and 8 h, and the presence of food after exposure on the treated concrete versus no food after exposure. Each of four replicates comprised five concentrations (4 rates of the insecticide + an untreated control) × three exposure periods × two food conditions after exposure, or 30 total exposure arenas. A Badger 100 artist's airbrush (Franklin Park, IL, USA) was used to spray the individual formulated sprays of chlorfenapyr directly onto the concrete arenas, which were then allowed to dry for several hours on a laboratory counter. The concrete arenas comprising the control replicates for each of the four separate treatments were sprayed with the same volume of tap water, that is, 0.3, 0.6, 0.9, and 1.2 mL depending on the companion insecticide treatment. After the dishes had dried, 10 1−2-week-old adult *T. castaneum* were exposed for the respective time periods, and then transferred to new Petri dishes lined with filter paper. Half of these dishes contained 500 mg of a standard flour-rearing media. Replicates were done as blocks at 2-day intervals, and all of the *T. castaneum* were obtained from pesticide-susceptible colonies maintained at the USDA-ARS-Grain Marketing and Production Research Center, Manhattan, KS, USA. This colony had been in culture since approximately 1958 and occasionally supplemented with *T. castaneum* collected from field sites. These colonies were reared on a mixture of 95% whole wheat flour and 5% brewer's yeast, 27 °C, 60% RH in continual darkness. The actual spraying was done at room temperature, which was ≈ 25 °C.

The condition of the beetles was assessed after the exposed *T. castaneum* were transferred to the new Petri dishes containing either flour or no flour (time 0). Beetles that were immobile and unresponsive when touched with a probe were considered dead, while beetles that were upright, mobile, and moving normally were considered to be "surviving". These observations were repeated daily for 7 days, or eight total assessments including the one done at time 0. The variable of analysis was survival, as described in the preceding sentence.

Data were analyzed as a multi-factorial experiment with exposure time, presence of food, and concentration as the main effects, and with days post-treatment as a repeated measure because all of the daily observations were done on the same group of insects. Control survival was 97.7% ± 0.4% in all replicates for the 7-day post-exposure assessments. No corrections for control mortality were necessary and the controls were eliminated from the statistical analysis. Few beetles could be accurately classified as knocked down, and since mortality and survival were reciprocal, only data for survival are presented. All analysis was done using the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, 2001), and raw data were converted to percentages and transformed by square root to account for non-heterogeneity of variances.

Data were analyzed using the General Linear Models Procedure of SAS to determine significance of main effects, the repeated measure day post-treatment, and the interactions between the main effects and the repeated measure. Significance between the two food treatments was determined using the *t*-test Procedure of SAS (PROC *T*-test, $P < 0.05$). Survival for each of the concentration levels and exposure intervals, which was the dependent variable y, was plotted with day post-treatment as the independent variable x. Equations were fit to the data using table curve 2D software (SPSS, Chicago, IL, USA) to determine the best linear or non-linear equation that fit the data, based on the R^2 value. The software calculates the R^2 value of the selected equation and also the maximum R^2 of any model which could be fit to the data set. This approach is based on curve-fitting procedures for biological data (Draper &

Smith, 1981), and has been used in previous studies (Arthur, 2008). The plotted equations were then graphed using Sigma Plot (SPSS, Chicago, IL, USA).

Results

Main effects exposure period and the presence of a food source were significant at $P < 0.01$ ($F = 17.9$, df = 2,64; $F = 135.4$, df = 1,64; respectively), as was the repeated measure time (the 0−7-day post-exposure assessments, $F = 154.4$; df = 7,630). However; the actual concentration of the insecticide (4 different volume application rates of chlorfenapyr) was not significant ($F = 0.9$, df = 3,64 $P = 0.45$). All interactions were significant at $P < 0.01$ except for those involving concentration as one of the factors ($P \geqslant 0.05$).

Data were then analyzed by concentration, exposure period, and post-treatment assessment, with the presence of food as the variable of interest, using a *t*-test analysis (SAS, 2001). When beetles were exposed for 2 h on concrete treated with chlorfenapyr at 0.275 and 0.55 g [AI]/m², there were no significant differences in survival with or without food at any of the post-treatment assessments (Fig. 1A,B). As the application rate increased to 0.825 g [AI]/m², survival with food was greater than survival without food at 3 days post-exposure and afterwards until the completion of the 7-day post-exposure period (Fig. 1C). At the highest concentration of 1.1 g [AI]/m², survival with food was greater than survival without food from day 2 post-exposure onward (Fig. 1D). As the exposure interval was increased to 4 h, there was a sharp decrease in survival without food, while survival with food remained at a high level (Fig. 2A−D). At all but the highest concentration (Fig. 2D), survival with food was greater than survival without food beginning at 1 day post-treatment. Results for the 8-h exposures showed some slight inconsistencies. At the lowest concentration of 0.275 g [AI]/m², survival in the presence of food at the post-treatment intervals ranged from 45.5% to 83.0%, and was greater than survival without food beginning at 2 days post-treatment (Fig. 3A). As the concentration increased to 0.55 and 0.825 g [AI]/m², survival with food decreased, resulting in less significant difference at the post-exposure intervals (Fig. 3B,C). However, at the highest concentration of 1.1 g [AI]/m² (Fig. 3D), survival with food increased, as opposed to an expected decrease consistent with the results for Fig. 3B,C. At 1.1 g [AI]/m², significant differences (Fig. 3D) between beetles given food versus those without food began occurring at 2 days post-treatment.

Survival at all exposure periods and concentrations when the beetles were given food generally did not decline during the post-exposure period of 7 days, except for the results depicted in Fig. 3B,C. Therefore, only survival without food was further analyzed by fitting equations to the data, by concentration and exposure interval (Fig. 4A−D and Table 1). The curve-fit equations were exponential of the form $y = a + b*\exp(-x/c)$, except for the linear equations for 2- and 4-hour exposures at 0.275 g [AI]/m² and 0.55 g [AI]/m², respectively. In these equations, y is survival and x is day post-exposure. The values for R^2 and the maximum R^2 that could be fit to any model (Table 1) are an indication of the fit of the equations.

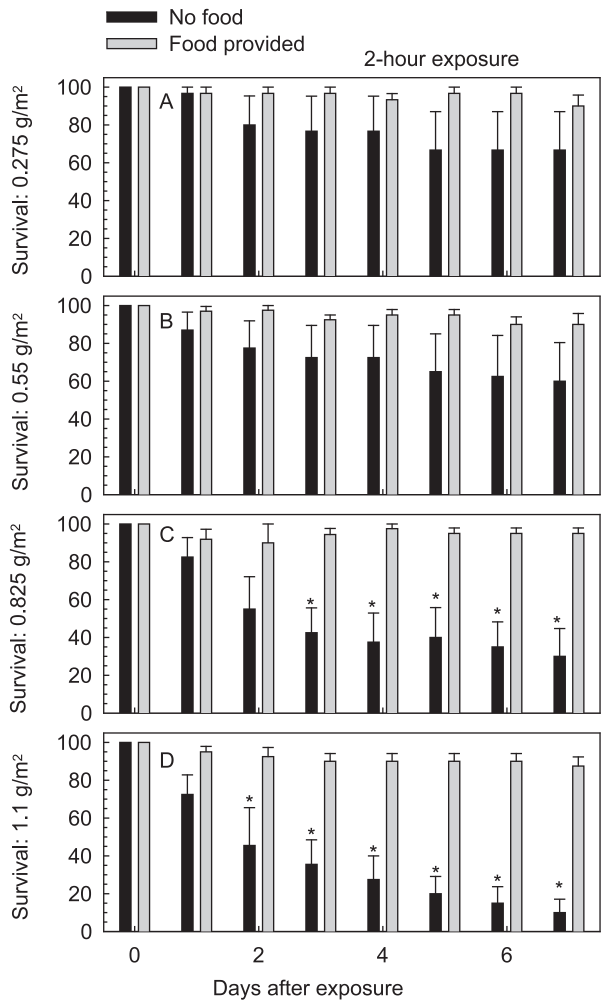

Fig. 1 Survival (%, means ± SE) of adult *Tribolium castaneum* exposed for 2 h on concrete treated with 0.275 (A), 0.55 (B), 0.825 (C), and 1.1 (D) g active ingredient [AI]/m² of chlorfenapyr and held for 0−7 days with or without food. Means at each of the daily observations for survival with food versus survival without food denoted with an asterisk are significantly different ($P < 0.05$, PROC *t*-test, SAS Institute).

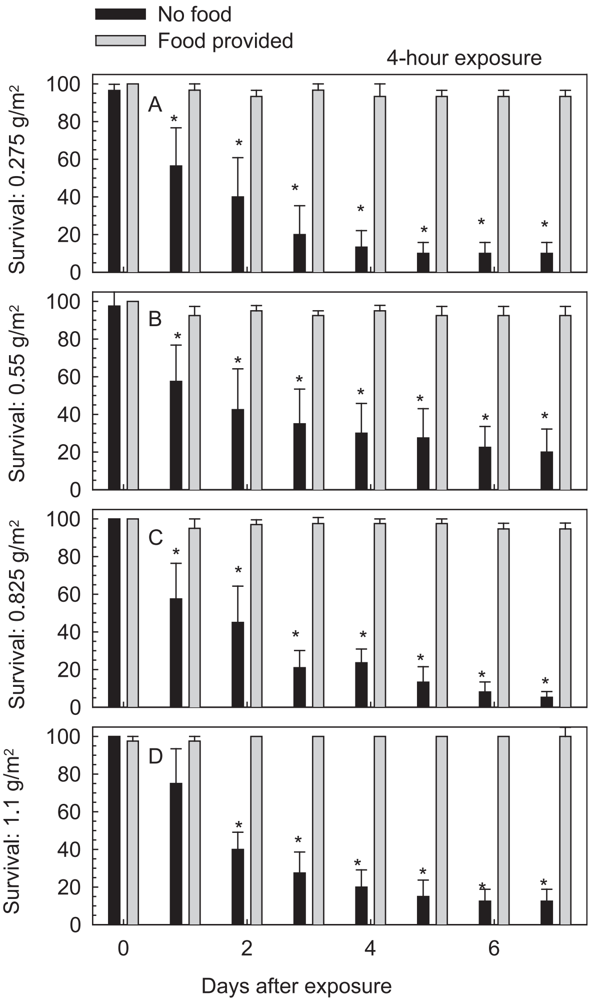

Fig. 2 Survival (%, means ± SE) of adult *Tribolium castaneum* exposed for 4 h on concrete treated with 0.275 (A), 0.55 (B), 0.825 (C), and 1.1 (D) g active ingredient [AI]/m^2 of chlorfenapyr and held for 0−7 days with or without food. Means at each of the daily observations for survival with food versus survival without food denoted with an asterisk are significantly different ($P < 0.05$, PROC *t*-test, SAS Institute).

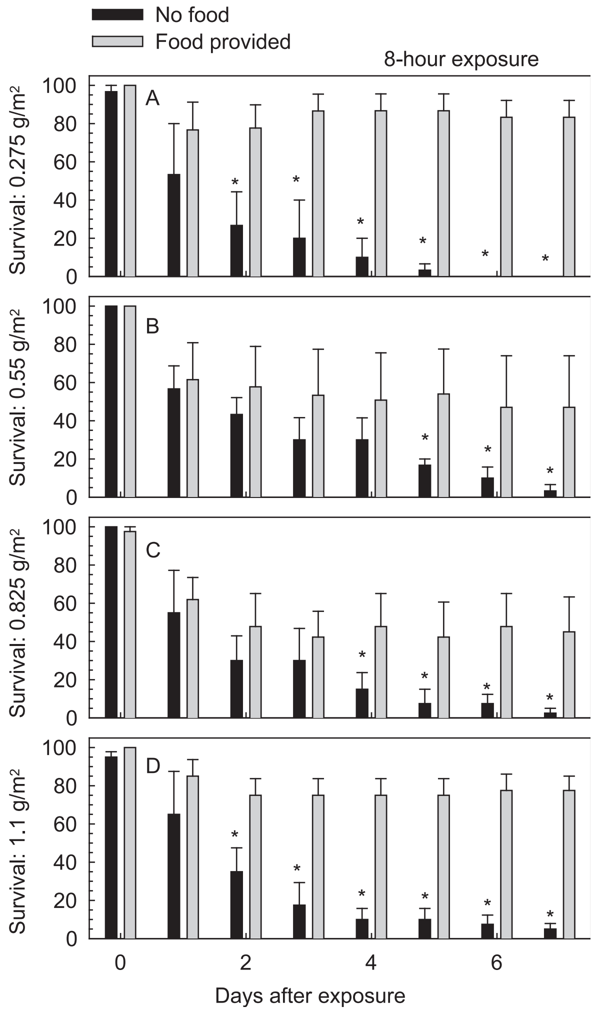

Fig. 3 Survival (%, means ± SE) of adult *Tribolium castaneum* exposed for 8 h on concrete treated with 0.275 (A), 0.55 (B), 0.825 (C), and 1.1 (D) g active ingredient [AI]/m^2 of chlorfenapyr and held for 0−7 days with or without food. Means at each of the daily observations for survival with food versus survival without food denoted with an asterisk are significantly different ($P < 0.05$, PROC *t*-test, SAS Institute).

A separate analysis of variance (ANOVA) was also run on the portion of the data set where the beetles were not given food after exposure, with concentration and exposure period as main effects, with time as the repeated measure. The results showed exposure period and time were significant at $P < 0.01$ ($F = 9.1$, df = 2,32; $F = 151.2$, df = 7,224; respectively), but concentration was not ($F = 1.1$, df = 3,32, $P = 0.35$), even though survival at the 2-h exposures appeared to decrease as concentration increased (Fig. 4A−D). This discrepant result can partially be explained by accounting for time as a repeated measure, which reduces the denominator degrees of freedom, and the inherent variation in the data. In contrast, the curve-fit equations in Table 1 and the plots for survival in Fig. 4 for the 4- and 8-h exposure were similar for each of the four concentrations. This would appear to be an indication that the time *T. castaneum* was exposed on the treated concrete was a more important determinant of survival than the concentration of chlorfenapyr that was applied. However, the variation in survival inherent within the data set, along with inconsistencies previously noted, could have masked the effects of increasing concentration

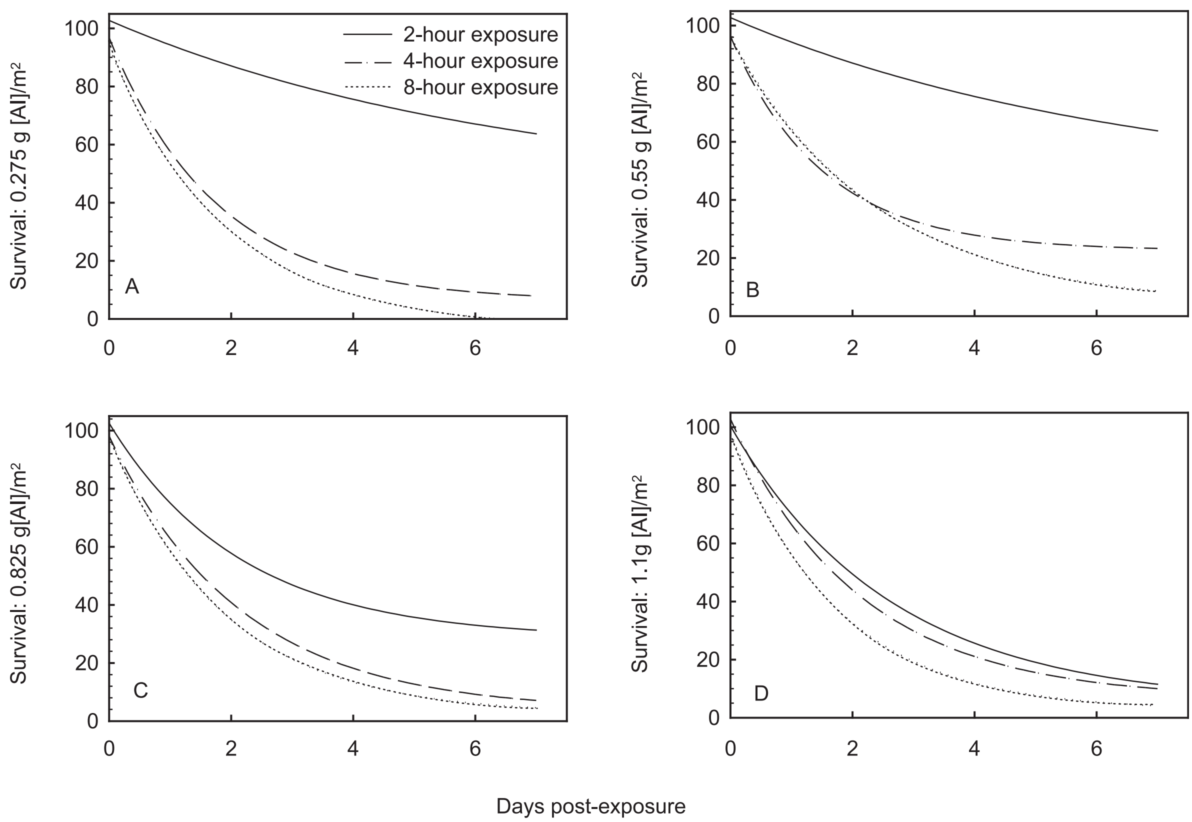

Fig. 4 Curve-fit equations (equation parameters from Table 1) for percentage survival of adult *Tribolium castaneum* exposed for 2, 4, or 8 h on concrete treated with 0.275 (A), 0.55 (B), 0.825 (C), and 1.1 (D) g [AI]/m^2 of chlorfenapyr and held for 0−7 days without food.

Table 1 Parameters for curve-fit equations plotted in Fig. 4 for survival of *Tribolium castaneum* 0−7 days† after exposure for 2, 4, or 8 h on concrete treated with chlorfenapyr at 0.275, 0.55, 0.825, and 1.1 g active ingredient [AI]/m^2. Also shown is the R^2 value for the equation and the maximum R^2 (max R^2) of any model that could be fit to the data.

Exposure (h)	g[AI]/m^2	A	B	C	R^2	max R^2
2	0.275‡	99.6 ± 9.2	5.5 ± 2.2		0.24	0.26
	0.550‡	93.1 ± 9.6	5.3 ± 2.3		0.15	0.16
	0.825	28.4 ± 12.7	74.1 ± 14.9	2.2 ± 1.2	0.49	0.51
	0.110	4.9 ± 14.0	95.5 ± 14.4	2.6 ± 1.1	0.68	0.68
4	0.275	6.1 ± 8.8	90.7 ± 12.4	1.7 ± 0.6	0.72	0.73
	0.550	22.5 ± 9.8	73.8 ± 15.7	1.5 ± 0.8	0.43	0.44
	0.825	3.4 ± 10.1	94.7 ± 12.0	2.1 ± 0.7	0.71	0.72
	0.110	6.4 ± 9.2	96.5 ± 10.1	2.1 ± 0.7	0.75	0.76
8	0.275	−3.2 ± 10.4	96.4 ± 13.8	1.9 ± 0.7	0.71	0.72
	0.550	3.8 ± 9.1	92.5 ± 9.9	2.4 ± 0.7	0.84	0.86
	0.825	2.4 ± 9.1	96.0 ± 12.3	1.8 ± 0.6	0.69	0.70
	0.110	2.4 ± 7.6	95.9 ± 10.9	1.7 ± 0.5	0.74	0.76

†Beetles were not given food after exposure or during the 7-day post-treatment period.
‡Curve-fit equations $y = a-b(x)$, all others are of the form $y= a + b*\exp(-x/c)$.

within any of the exposure intervals, particularly the shortest interval of 2 h.

Discussion

The decreased survival of *Tribolium castaneum* exposed to chlorfenapyr in the presence of food is similar to results reported for the same strain of *T. castaneum* exposed to the pyrethroid cyfluthrin (Arthur, 1998a, 1998b, 2000a) and the inert dust diatomaceous earth (DE) (Arthur, 2000b). However, the results in the current study with chlorfenapyr appear to show a much greater increase in survival in the presence of food compared to the results for the earlier studies with cyfluthrin and DE. Chlorfenapyr is an insecticidal pyrrole, and the primary mode of action is to affect oxidative phosphorylation in the mitochondria, which will eventually result in the death of the cell through inhibition of ATP synthesis (Hunt, 1996; Mascarenhas & Boethel, 1997; McLeod *et al.*, 2002). This mode of action differs from that of a conventional neurotoxin, and mortality of *T. castaneum* as a result of exposure to chlorfenapyr is not immediate but is delayed for several days after the initial exposure (Arthur, 2008). The presence of food apparently provided enough nutrition to counteract the effects of exposure, particularly for the 2- and 4-h exposure periods. The impact of the food was somewhat less at the 8-h exposure period, as shown by survival ranging from 45% to 83% at the 7-day post-treatment assessments, depending on the actual concentration that was applied to the concrete treatment arenas.

There are a number of studies that document effectiveness of contact insecticides when stored-product insects are exposed on treated surfaces for fixed time intervals (Arthur, 1999; Toews *et al.*, 2003). Results for the comparatively short time intervals assessed in the current study with chlorfenapyr would seem to indicate that it would be a valid addition to pest management programs to control *Tribolium* spp. in mills and warehouses. However, in actual field applications, residual insecticides can be applied to limited areas such as spots, cracks and crevices, and peripheral areas along the perimeter of a structure (Toews *et al.*, 2003). Therefore, the amount of time that adult *T. castaneum* infesting field sites would spend on a surface treated with chlorfenapyr (or any other residual contact insecticide), is an unknown variable. The larger the treated area, the more likely an adult would be exposed to the insecticide, but the resident populations are often hidden in refugial areas, and may escape exposure (Barson, 1991). If these refugial areas contain food, the limited exposure time on the treated surface, coupled with the availability of the food resource, could compromise the effectiveness of the insecticidal application. Toews *et al.* (2005) conducted simulated field studies by creating shelf structures inside small sheds and infesting areas underneath the shelves with different life stages of *T. castaneum* in flour refuges, then subsequently applying cyfluthrin in a banding pattern on the floor around the shelves. Although adult *T. castaneum* were killed by apparent exposure to the cyfluthrin residues, overall populations in the refugial areas underneath the shelves did not decrease significantly from the populations that were in the same areas in an untreated shed. Either the food resource could have mitigated the effects of adult exposure to the cyfluthrin residues or the surviving adults were able to more fully exploit the food resource, thereby enabling the infestation to continue even though adults were being killed by exposure to the cyfluthrin residues.

Increasing sanitation efforts in milling and warehousing facilities could eliminate refugia where *T. castaneum* populations can develop and spread (Campbell & Arthur, 2007), and also increase the effectiveness of chlorfenapyr and other insecticides used in the pest management program. Monitoring pest populations would also be of great benefit in pest management programs that utilize insecticides as a control strategy (Campbell *et al.*, 2002; Campbell & Arbogast, 2004). Spatial analysis is an effective method for mapping the distribution and spread of stored-product insects (Brenner *et al.*, 1998; Arbogast *et al.*, 2000, 2002), which could provide a means to specifically target insecticidal applications (Toews *et al.*, 2005). A more precise targeting and application of insecticides could minimize the ability of mobile adults such as *T. castaneum* to escape exposure on the treated surface. However, the importance of the sanitation and cleaning in mills, warehouses, and other environments that harbor *T. castaneum* and other stored-product insects should be emphasized as an integral component of the management program. Although chlorfenapyr may be a viable option for insect control, the presence of food will greatly reduce the effectiveness of this insecticide. Reducing extraneous food sources inside structural environments that contain stored-product insects should enhance the effectiveness of any insecticide used in the management program.

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or trade name does not constitute a recommendation or endorsement by the US Department of Agriculture.

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